AN INVESTIGATION OF THE SOOTY MOULDS OF NEW SOUTH WALES. II.

AN EXAMINATION OF THE CULTURAL BEHAVIOUR OF CERTAIN SOOTY MOULD FUNGI.

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(Fifty-nine Text-figures.)

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Introduction.

In a previous paper (Fraser, 1933) the composition of the sooty moulds of New South Wales was described. It was pointed out that the sooty mould forming fungi belong to three systematic divisions, the Capnodiaceae, the Atichiaceae and the Fungi Imperfecti. It is common to find several different fungi taking part in the formation of a single sooty mould. It was further shown that the fungi forming sooty moulds could be grouped according to their modes of occurrence into three divisions, perennials, ephemerals, and accidentals. The perennials, which include the Capnodiaceae and Atichiaceae, together with *Dematium pullulans* and *Cladosporium herbarum*, have a dark-brown or olive-green mycelium which is resistant to heat and desiccation. The ephemerals, which include members of the Fungi Imperfecti such as *Alternaria*, *Penicillium*, *Epicoccum* and *Asbolisia* spp., tide over adverse periods by means of their resistant spores. The accidentals, which include *Fusarium* spp., *Mucor* spp., bacteria, etc., disappear completely from the sooty mould during adverse weather conditions, and their reappearance is due to accidental infection.

Sooty moulds which appear on annual plants following attack by aphis are composed principally of *Cladosporium*, *Dematium* and a number of ephemerals. Similar sooty mould associations often develop on trees and shrubs attacked by scale insects, but usually they are replaced after a short time by associations in which one or more members of the Capnodiaceae are present.

As the commencement of an inquiry into the physiology of the members of the sooty mould flora, experiments have been made to investigate the ability of a selected number of these fungi to utilize a variety of nutrient compounds.

The composition of the honey dew on which sooty moulds grow (see Arnaud, 1911) is dextrin, gums, etc. In addition mineral material in the form of dust may be available to the sooty mould fungi. The natural medium, therefore, consists of complex carbohydrate and protein materials with various mineral salts.

It is noticeable that common saprophytes such as *Penicillium expansum* and *Cladosporium herbarum* occur to a greater or lesser degree in sooty mould formations. The highly specialized members of the Capnodiaceae on the other hand are not found elsewhere. Although they must possess the ability to utilize complex organic food materials, they are not found in competition with decay-causing fungi except on leaves covered with honey dew.

The sooty mould associations appear, therefore, to include fungi definitely adapted for the habitat in which they occur. It was with the object of finding out some of the physiological characteristics of the group that the experiments here reported were made. The fungi selected were grown on a variety of different food materials in order to find out if it were possible to group them according to their ability to utilize certain classes of organic and inorganic compounds. If this were possible it would indicate a basis for the interpretation of the distribution of these fungi.

The group of fungi chosen for this work includes representatives of all the classes of sooty mould fungi as defined in a previous paper (Fraser, 1933) as follows: *Botrytis cinerea* Pers., *Penicillium expansum* Thom., *Asbolisia* sp., *Cladosporium herbarum* Link., *Dematium pullulans* de Bary, *Caldariomyces* sp. (near *C. fumago* Woron.), *Capnodium* sp., 2 strains, *Microxyphium* sp. A, 2 strains, *Microxyphium* sp. B, *Microxyphium* sp. C.

Microxyphium, *Capnodium* and *Caldariomyces* are representatives of the permanent Capnodiaceae flora. The species of *Microxyphium* grow slowly in culture, *Capnodium* and *Caldariomyces* grow more quickly. *Dematium* and *Cladosporium* are important constituents of both perennial and annual moulds, *Cladosporium* being a widespread saprophyte. Both grow rapidly in culture. *Asbolisia* belongs to the ephemeral class of sooty mould fungi; it is fairly common and grows very rapidly in culture. *Penicillium* is a common saprophyte and a member of the ephemeral class of sooty mould fungi. It was included partly as a control, because of its cosmopolitan nature. *Botrytis* was also included as a control. It is a common saprophyte of decaying vegetable matter, but is not present in the sooty moulds of New South Wales.

Cultural Work.

The effects of the various types of food materials used in the investigations on the growth of the fungi were studied by measuring the amount of growth made on agar containing the different nutrients under investigation.

Sterilized Petri dishes of 9 cm. diameter were poured with 10 c.c. of agar of the appropriate composition, so that the resulting layer of agar was comparatively thin. The experiments were carried out in triplicate. All cultures were incubated in darkness at 25° C. throughout the investigation, so that as far as possible conditions would be comparable between the different sets of experiments. The dishes were inoculated with the fungus to be examined, and the diameter of the colony measured three times weekly. The hydrogen-ion concentration of the media was adjusted to pH 5·5 throughout.

There are a number of objections to the method of determining the growth of fungi in culture by the measurement of the diameter of the colonies. For example, growth on one medium may be rapid, but the resulting colony thin and straggling, whereas on a different medium the increase in diameter may be slower, but the growth is more efficient as shown by the production of spores. It has been maintained, therefore, that the measurement of dry weight gives more reliable results.

In the present investigation allowances have been made, as far as possible, in the statement of conclusions, for the different types of growth. The method of examination of growth by measurement of the diameter of the colony was used because, as well as the final comparison of growth on different types of agars, the growth rate of a fungus on any one agar could also be studied. Indications of staling phenomena, should they occur, could thus be detected.

Growth on Carbohydrate Media.

Waksman's peptone agar was used throughout, with a 2% concentration of the carbohydrate to be tested. It was made up as follows: peptone 0·5%, potassium dihydrogen phosphate 0·1%, magnesium sulphate 0·05%, carbohydrate 2%, agar 1·5%.

The following carbohydrates were used:

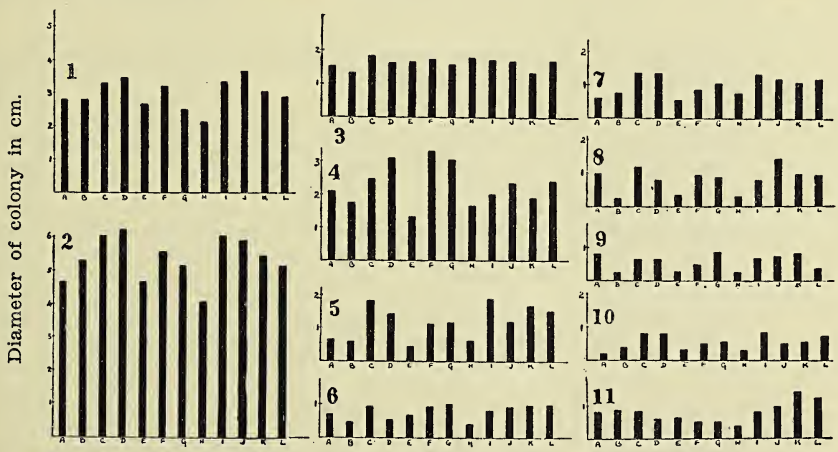
(i) Sugars.—(*a*) Monosaccharides. Pentoses ($C_5H_{10}O_5$): arabinose, xylose; hexoses ($C_6H_{12}O_6$): dextrose, levulose, mannose.
(*b*) Disaccharides ($C_{12}H_{22}O_{11}$): sucrose, maltose, lactose.
(*c*) Trisaccharide: raffinose.

(ii) Polysaccharides: inulin, dextrin, starch.

(i) *Sugars.*—The comparative amounts of growth made on the different agars by the selected fungi are shown in Text-figures 1–11. Except in the cases of *Microxyphium* spp. A and B, the text-figures show the diameters of the colonies 7 days after inoculation. The species of *Microxyphium* have a very slow growth rate, the colony 7 days after inoculation being very small. It was therefore found advisable in this case to give the amount of growth after 21 days. No text-figures are included to show the amount of growth made by *Botrytis cinerea* since, after 7 days, the fungus colony usually covered the whole area of the Petri dish.

A summary of the results obtained, with special regard to the types of growth and amount of spore production, is given in Table 1.

It appears from this table that there are a number of differences between the two strains of *Capnodium* sp., as shown by their reactions on similar media. The chief difference is that strain 1 grows poorly on dextrose agar, whereas strain 2 grows well. The growth rate of strain 2 on all media is slightly higher than that of strain 1 (Text-figs. 6–7).



Text-figs. 1-11.—Tables to show the amount of growth made on different carbohydrate media by sooty mould fungi. (A, arabinose; B, xylose; C, levulose; D, dextrose; E, mannose; F, sucrose; G, maltose; H, lactose; I, raffinose; J, inulin; K, starch; L, dextrin.) 1. *Penicillium expansum*; 2. *Asbolisia* sp.; 3. *Cladosporium herbarum*; 4. *Dematium pullulans*; 5. *Caldariomyces* sp.; 6, 7. *Capnodium* sp.; 8. *Microxyphium* sp. A, strain 1; 9. *Microxyphium* sp. A, strain 2; 10. *Microxyphium* sp. C; 11. *Microxyphium* sp. B.

TABLE 1.

Fungus.	Monosaccharides.			
	Pentoses.		Hexoses.	
	Arabinose.	Xylose.	Dextrose.	Levulose.
Botrytis cinerea ..	Growth and spore production poor.		Growth and spore production very good.	
Penicillium expansum	Growth and spore production medium.		Growth and spore production very good.	
Asbolisia sp. ..	Growth medium, pycnidium production poor.		Growth and pycnidium production very good.	
Cladosporium herbarum ..	Growth and spore production medium.		Growth and spore production good.	Growth and spore production very good.
Dematium pullulans	Growth medium, colony thin, olive-green.	Growth medium, colony dark, slimy due to numerous conidia.	Growth good, colony olive-green.	Growth medium, colony olive-green.
Caldariomyces sp...	Growth very poor, spore production medium.		Growth and spore production very good.	Growth and spore production good.
Capnodium sp. strain 1.. ..	Growth and pycnidium production medium.		Growth and pycnidium production poor.	Growth and pycnidium production very good.
Capnodium sp. strain 2.. ..	Growth and pycnidium production poor.	Growth and pycnidium production poor.	Growth and pycnidium production good.	
Microxyphium sp. A, strain 1.. ..	Growth and pycnidium production medium.	Growth and pycnidium production very poor.	Growth and pycnidium production medium.	Growth and pycnidium production good.
Microxyphium sp. A, strain 2.. ..	Growth and pycnidium production medium.	Growth and pycnidium production very poor.	Growth and pycnidium production medium.	Growth and pycnidium production good.
Microxyphium sp. B	Growth good.	Growth good.	Growth medium.	Growth good.
Microxyphium sp. C.	Growth and pycnidium production poor.	Growth and pycnidium production very poor.	Growth and pycnidium production good.	

TABLE 1.

	Disaccharides.			Trisaccharide.	Text-fig.
Mannose.	Sucrose.	Maltose.	Lactose.	Raffinose.	
Growth and spore production very good	Growth and spore production very good.		Growth and spore production poor.	Growth and spore production poor.	
Growth and spore production medium.	Growth and spore production good.	Growth and spore production medium.	Growth and spore production poor.	Growth and spore production good.	1
Growth and pycnidium production medium.	Growth and pycnidium production good.	Growth and pycnidium production medium.	Growth and pycnidium production poor.	Growth and pycnidium production very good.	2
Growth and spore production good.	Growth and spore production very good.	Growth and spore production good.	Growth and spore production very good.	Growth and spore production very good.	3
Growth very poor, colony white.	Growth very good, colony olive-green.	Growth medium, colony olive-green.	Growth poor, colony white.	Growth rather poor, colony white.	4
Growth and spore production medium.	Growth and spore production medium.		Growth poor, spore production good.	Growth and spore production very good.	5
Growth and pycnidium production rather poor.	Growth and pycnidium production good.		Growth and pycnidium production medium.	Growth and pycnidium production good.	6
Growth and pycnidium production poor.	Growth and pycnidium production medium.			Growth and pycnidium production good.	7
Growth and pycnidium production poor.	Growth and pycnidium production good.		Growth and pycnidium production poor.	Growth and pycnidium production fairly good.	8
Growth and pycnidium production poor.	Growth and pycnidium production medium.	Growth and pycnidium production good.	Growth and pycnidium production poor.	Growth and pycnidium production fairly good.	9
Growth poor.	Growth medium.		Growth poor.	Growth good.	11
Growth and pycnidium production medium.	Growth and pycnidium production good.		Growth poor, pycnidium production medium.	Growth and pycnidium production good.	10

The differences in reaction to similar culture media between strains 1 and 2 of *Microxyphium* sp. A (Text-figs. 8–9) are only of minor importance, the chief being the greater growth rate of strain 1.

All the sugars used in this experiment are utilized to a greater or lesser degree by the fungi under investigation. On the whole the pentoses are not so satisfactory for the maintenance of growth and reproduction as are the hexoses. Of the hexoses, mannose is generally poorly utilized, whereas dextrose and levulose are uniformly very satisfactory. Of the disaccharides, sucrose and maltose are moderately satisfactory, and lactose poor. The trisaccharide raffinose is unsatisfactory for *Dematium pullulans* (Text-fig. 4) and *Botrytis cinerea*, but is otherwise well utilized, especially by the members of the Capnodiaceae.

The growth reactions of the Capnodiaceae to the various sugars are strikingly similar (Text-figs. 5–11). It is noticeable that the two widespread saprophytes, *Penicillium expansum* (Text-fig. 1) and *Cladosporium herbarum* (Text-fig. 3) show less difference in growth on the different sugars than do the other fungi. This ability to utilize a wide range of food materials is no doubt responsible for their cosmopolitan distribution. On the other hand the growth reactions of *Dematium pullulans* (Text-fig. 4), which is also a widespread saprophyte, are in striking contrast with those of *Penicillium* and *Cladosporium*. In the case of *Dematium*, and to a lesser extent in the Capnodiaceae, the growth on the various media used shows a great variation. Instead of a more or less uniform growth on all the media, growth on some of the sugars may be very good, whilst other sugars may scarcely be utilized at all, as with *Dematium* on mannose.

On the whole the growth of the members of the Capnodiaceae is very slow compared with that of the other fungi investigated.

The mould *Botrytis cinerea* shows the least ability to utilize the range of carbohydrates, which is contrary to what would be expected when its widespread occurrence is considered.

The growth rates of *Penicillium* and *Caldariomyces* on a representative number of the agars used are shown in Text-figures 12 and 13. They are typical of the fast and slow growing fungi respectively.

Brown (1923) has pointed out that in culture media the growth of a fungus is at first slow, but increases until a maximum is reached, at which it remains steady, or from which it subsequently declines. A declining growth rate is due to staling of the medium by materials produced by the growth of the fungus.

Text-figure 12 shows that there is no evidence of staling by *Penicillium* during the period in which the fungus was under investigation, even in the case of sugars which were not very satisfactory for growth, such as lactose and mannose.

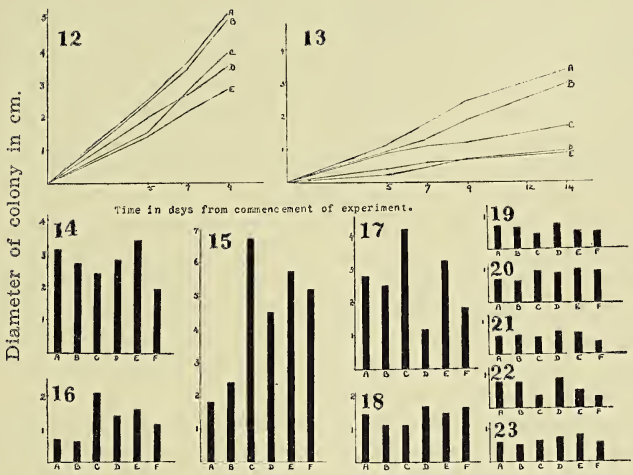
The slight flattening of the curves of *Caldariomyces* shown in Text-figure 13 indicates slight staling, especially in the cases of xylose and mannose.

(ii) *Polysaccharides.*—The amount of growth made on polysaccharide media is shown in the preceding figures. In all cases good growth was made, comparable with or better than that made on the sugar media. Inulin is found to be slightly less favourable than starch or dextrin for the growth of members of the Capnodiaceae, but for the other fungi it is quite satisfactory. The growth rates of all the fungi are uniform on the polysaccharide media, and there is no evidence of staling during the period when the cultures were under investigation.

From the above results it is not possible to find any definite relationship between the distribution of sooty mould fungi and their ability to utilize various

carbohydrates as a source of food. The two most widespread mould fungi, *Penicillium* and *Cladosporium*, show the greatest ability to utilize a wide range of carbohydrates.

Farries and Bell (1930), working with *Nematospora* and allied fungi, found that growth on media containing the pentoses arabinose and xylose was negligible. Glucose, fructose and mannose, on the other hand, were very favourable. Sucrose and maltose were favourable, but growth on lactose was negligible. One species of *Nematospora* made good growth on starch, the others making slight but quite



Text-fig. 12.—Graph to show the growth rate of *Penicillium expansum* on a number of carbohydrate media. (A, inulin; B, dextrose; C, arabinose; D, mannose; E, xylose.)

Text-fig. 13.—Graph to show the growth rate of *Caldariomyces* sp. on a number of carbohydrate media. (A, levulose; B, raffinose; C, inulin; D, mannose; E, xylose.)

Text-figs. 14-23.—Tables to show the amounts of growth made on different nitrogen compounds by sooty mould fungi. (A, ammonium sulphate; B, ammonium nitrate; C, potassium nitrate; D, sodium nitrate; E, peptone; F, asparagin.) 14. *Penicillium expansum*; 15. *Asbolisia* sp.; 16. *Cladosporium herbarum*; 17. *Dematium pullulans*; 18. *Caldariomyces* sp.; 19, 20. *Capnodium* sp.; 21. *Microxyphium* sp. A, strain 1; 22. *M.* sp. B; 23. *M.* sp. C.

definite growth, indicating the presence of diastase. Norman (1930) found that certain soil fungi showed greater ability to utilize hexose than pentose sugars.

It is apparent that the sooty mould fungi investigated in the present work have a greater ability to utilize a wide range of carbohydrate materials than have parasitic species such as *Nematospora*.

Growth on Nitrogen Media.

In order to investigate the ability of the sooty mould fungi to utilize various nitrogen compounds the following inorganic and organic sources of nitrogen were used. Inorganic: ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate. Organic: peptone, asparagin.

Agars were made up according to the formula of Farries and Bell (1930) as follows: agar 2%, dextrose 2%, potassium dihydrogen phosphate 0·5%, magnesium sulphate 0·25% and the nitrogen compound in sufficient quantity to yield 0·3% nitrogen.

The amount of growth made by each fungus on the different nitrogen compounds is shown in Text-figures 14–21. Except in the case of *Microxyphium* spp. A and B, for which the figures indicate growth after 21 days, the diameter of the fungal colony after 7 days is given. Curves to show the individual growth rates of a representative set of fungi, *Asbolisia*, *Penicillium*, *Cladosporium*, *Dematium* and *Caldariomyces*, are also shown. From these figures it can be seen that there is a great variety of reactions to the different compounds.

Botrytis cinerea.—Growth is feeble on the ammonium compounds and on KNO_3, moderately good on $NaNO_3$, and good on peptone and asparagin.

Penicillium expansum (Text-fig. 14).—Growth is satisfactory on all compounds except asparagin. Peptone and NH_4SO_3 give the best results. Although at first growth on asparagin is not much less than on other media (Text-fig. 24), the growth rate falls off rapidly and ceases altogether by the ninth day (as shown by the flattening of the curve for asparagin in Text-fig. 24). Staling is therefore indicated. Slight staling is also indicated by the declining rate of increase shown by the curve for NH_4NO_3. Very rapid growth in diameter takes place on KNO_3, but the colony is thin and the conidia sparse.

Asbolisia sp. (Text-fig. 15).—Growth is poor on ammonium salts. On KNO_3 growth is rapid but thin. On asparagin, though the growth is rapid, the colony is pale and uneven, and no pycnidia are produced. Peptone and $NaNO_3$ are very satisfactory. From a study of the growth rate of the fungus on the different media (Text-fig. 25), it is apparent that staling takes place on asparagin and ammonium salts. As is the case with *Penicillium*, the growth rate on KNO_3 is very fast, but the resulting colony is very thin.

Cladosporium herbarum (Text-fig. 16).—Growth is poor on ammonium salts, medium on asparagin, very good on KNO_3, and good on $NaNO_3$ and peptone. Text-figure 26 shows the growth rates on the various media. The curves for ammonium salts and for asparagin indicate that staling is strongly marked on these media.

Dematium pullulans (Text-fig. 17).—Growth is good on KNO_3 and peptone, and medium on the other compounds. $NaNO_3$ is rather unsatisfactory. The growth curve for asparagin (Text-fig. 27) indicates pronounced staling. Slight staling is also indicated by the curves representing growth on ammonium salts, peptone and $NaNO_3$.

Caldariomyces sp. (Text-fig. 18).—Growth and spore production are satisfactory on all media except asparagin, $NaNO_3$ being the most favourable. As shown in Text-figure 28, growth on asparagin is at first good, but staling becomes very pronounced. Staling is also indicated on KNO_3 and NH_4NO_3.

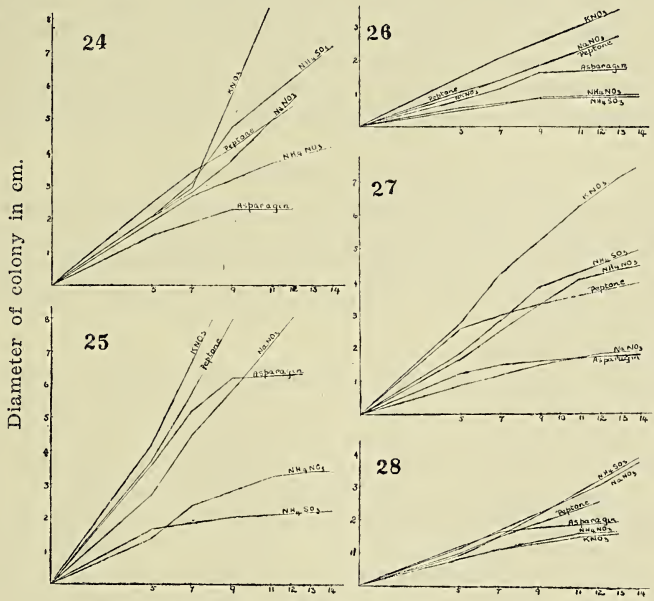
Capnodium sp. (strain 1, Text-fig. 19; strain 2, Text-fig. 20).—On the whole, growth is faster in strain 2 than in strain 1. Growth and spore production are best on $NaNO_3$ and peptone, but all media are fairly satisfactory. Staling is marked on asparagin.

Microxyphium sp. A (strain 1, Text-fig. 21).—Both strains react similarly. Growth and pycnidium production are best on $NaNO_3$ and peptone, medium on ammonium salts and KNO_3 and poor on asparagin. Staling is not shown because of the slow rate of growth.

Microxyphium sp. B (Text-fig. 22).—Growth is best on $NaNO_3$ and ammonium salts, poor on KNO_3 and asparagin, and medium on peptone. Staling is not shown.

Microxyphium sp. C (Text-fig. 23).—Growth is good on $NaNO_3$ and peptone, and rather poor on ammonium salts, KNO_3 and asparagin. Staling takes place on asparagin.

It will be apparent at once that the results obtained for growth on nitrogen compounds differ from those obtained for growth on carbohydrates. Growth on unfavourable nitrogen compounds is accompanied, especially in the case of the fast-growing fungi, by pronounced staling. On unfavourable carbohydrates, on the other hand, growth is slow, but staling, if shown at all, is not pronounced.



Text-figs. 24-28.—Graphs to show growth rates on different nitrogen compounds: 24. *Penicillium expansum*; 25. *Asbolisia* sp.; 26. *Cladosporium herbarum*; 27. *Dematium pullulans*; 28. *Caldariomyces* sp.

From this it may be concluded that certain carbohydrates are unsatisfactory because they cannot readily be utilized by the fungus, whereas, in the case of unfavourable nitrogen compounds, materials are produced as the result of the metabolism of the fungus which are inimical to further growth, and so cause staling.

Asparagin is unsatisfactory as a source of nitrogen for all fungi except *Botrytis cinerea*, and growth on this medium is accompanied by pronounced staling in all cases but that of *Botrytis*. The fact that *Botrytis* grows very quickly and is a non-staling type of fungus (Brown, 1923) may account for its good growth on asparagin, since staling is not shown by other fungi till after about 7 days, and by that time the growth of *Botrytis* has covered the area of the Petri dish.

Ammonium salts are unsatisfactory for *Cladosporium*, *Dematium* and *Asbolisia*, but are utilized moderately well by the Capnodiaceae, and very well by *Penicillium*.

The growth of the fungi on KNO_3 is generally thin. Peptone and $NaNO_3$ are the most universally satisfactory nitrogen compounds. $NaNO_3$ is very satisfactory for the Capnodiaceae, rather more so than peptone, but is very unsuitable for *Dematium pullulans*.

Penicillium has the ability to utilize the greatest range of nitrogen compounds, growing equally well on organic and inorganic media. *Cladosporium herbarum*, which, like *Penicillium*, has the ability to utilize a wide range of carbohydrate materials, is, on the other hand, fairly restricted in its growth on nitrogen compounds. It grows well only on $NaNO_3$, peptone and KNO_3. *Dematium pullulans* is also unable to utilize satisfactorily a wide range of nitrogen compounds.

Farries and Bell (1930) have found that peptone and, to a less degree, asparagin are utilized by *Nematospora*, but that KNO_3 and ammonium salts are of little value. On the whole, the sooty mould fungi show a considerably greater ability to utilize inorganic as well as organic nitrogen compounds than the parasitic *Nematospora*, but are less omnivorous than the widespread mould *Penicillium*.

The Carbohydrate-Nitrogen Ratio.

This section of the inquiry commenced with a study of the effects of increased concentrations of sugar on the growth and reproduction of the fungi under investigation. Since these were found to give suggestive results, the inquiry was extended to include other variations in the relative concentrations of carbohydrates and nitrogen.

The work falls into three sections:

(i). *Media of varying sugar concentration.*—Agar was made up in the manner described in the previous section, but the sugar concentration varied as follows: (*a*) low concentration 0·5%, (*b*) medium concentration 2%, (*c*) high concentration 10%. The nitrogen constituent was constant throughout, so that the media were of low, medium and high carbohydrate-nitrogen ratio respectively.

(ii). *Media of varying nitrogen concentration.*—Four sets of experiments were made: (*a*) with peptone dextrose agar, (*b*) with peptone maltose agar, (*c*) with $NaNO_3$ dextrose agar, (*d*) with $NaNO_3$ maltose agar. The sugar concentration was 2% throughout, and other constituents as before. The concentration of nitrogen varied as follows: (*a*) low concentration, nitrogen constituent to give 0·08% N, (*b*) medium concentration, nitrogen constituent to give 0·3% N, (*c*) high concentration, nitrogen constituent to give 1·2% N. Media were thus obtained with high, medium and low carbohydate-nitrogen ratios, differing in concentration from those in the previous section.

(iii). *Media of varying concentration of both nitrogen and sugar.*—Two sets of experiments were made, one in which peptone and the other in which $NaNO_3$ was the source of nitrogen. The agar was made up as previously, except that the concentrations of nitrogen and sugar were varied as follows: (*a*) low concentration, dextrose 0·5%, N compound to give 0·08% N, (*b*) medium concentration, dextrose 2%, N compound to give 0·3% N, (*c*) high concentration, dextrose 10%, N compound to give 1·2% N. In these media the carbohydrate-nitrogen ratio was therefore constant throughout.

All these experiments were not carried out simultaneously owing to lack of space, but since each set contained one identical group, viz., that in which the concentration of nitrogen and sugar was medium, it was possible to check them to see if comparable results were being obtained. It was found that the results for these groups were very close in each set, and therefore comparisons could be made.

(i) *Varying sugar concentration.*

In general, colonies on media of low sugar concentration (i.e., low carbohydrate-nitrogen ratio) grow fairly rapidly and fruit abundantly. Growth is thinner, however, than on media of medium concentration. Spore production is on the

whole slightly less than on media of medium concentration. On media of high sugar concentration spore production is slightly depressed, especially when peptone is the source of nitrogen.

The growth curve of *Cladosporium* on $NaNO_3$ agar is shown in Text-figure 29. This type of curve is also representative of *Penicillium*, *Capnodium* and *Microxyphium* sp. C. It is apparent that growth is most rapid on medium and slowest on concentrated media, but there is no sign of staling.

Text-figure 30 shows the growth curve of *Dematium pullulans* on peptone agar. This type of curve also illustrates the growth of *Asbolisia* sp. Growth on media with high concentration of sugar is extremely rapid, and more so on medium than on low concentrations. Text-figure 31 shows the growth curve for *Caldariomyces*. This is of the same general type as that of *Dematium pullulans*, but the contrast between the growth on the different concentrations is not so marked. Text-figure 32 shows the growth curve for *Microxyphium* sp. B. *Microxyphium* sp. A also exhibits this type of growth. Staling is indicated at high sugar concentration, and best growth takes place at medium concentration.

It appears from these results that the optimum sugar concentration for growth differs for each species. The majority of fungi grow and fruit best at medium concentration. *Botrytis*, *Dematium*, *Asbolisia* and, to a less extent, *Caldariomyces* are able to utilize to advantage much higher concentrations. Staling as a rule is not shown; it was noticed only with *Microxyphium* spp. A and B on media of high sugar concentration.

(ii) *Media of varying nitrogen concentration, and* (iii) *Media of varying concentration of both nitrogen and sugar.*

It will be convenient to deal with the results of the experiments carried out under sections (ii) and (iii) together. Curves indicating the growth rates of the fungi are given in the accompanying figures. The data concerning growth and spore production are summarized in Table 2. In some cases the growth curves for both maltose and dextrose agars are given. In other cases where the growth on the two sugars is very similar, one set of growth curves only is given. In the case of *Microxyphium* sp. A, the final amount of growth made after 21 days is given, since the growth rate of this fungus is very slow, and the growth curve does not show any points of special interest.

Botrytis cinerea.—At low concentrations of nitrogen, and in media in which the concentrations of nitrogen and sugar are both low, growth is thinner than at medium concentrations, and spore production is rather poorer. At high concentrations of nitrogen and sugar and of nitrogen alone growth is very rapid and spore production heavy, the resulting colonies being thicker and more darkly coloured than at lower concentrations.

Penicillium expansum.—Best growth is made on agars with medium concentrations of sugar and nitrogen. Growth is rather thin and spore production less on media of low concentration, especially when sugar as well as nitrogen is low. From an examination of Text-figure 33, which represents growth rates on dextrose and maltose $NaNO_3$ agars, it can be seen that there are indications of staling on maltose agar at high concentrations of $NaNO_3$. At this concentration spore production is quite normal. No staling is indicated at high concentrations of dextrose, but spore production is poor, and the growth rate is slower than at lower concentrations.

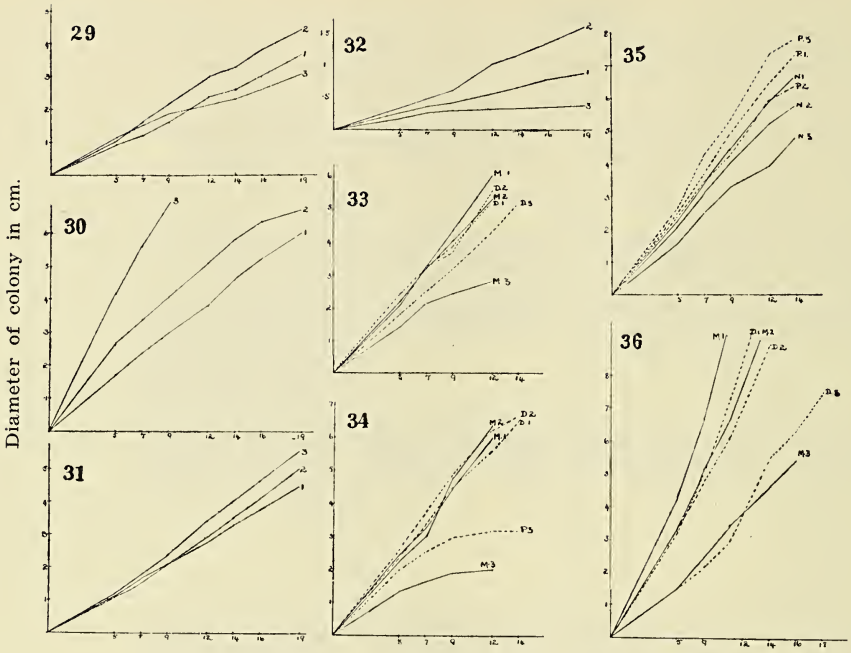
Staling is much more marked at high concentrations of peptone than of $NaNO_3$ (Text-fig. 34), and more so with maltose than with dextrose. If Text-figures 33

TABLE 2.

Fungus.	Carbohydrate-Nitrogen Ratio Medium.		
	Concentration low.	Concentration medium.	Concentration high.
Botrytis cinerea	Growth thin, spore production depressed.	Growth and spore production good.	Growth and spore production good.
Penicillium expansum ..	Growth thin, spore production medium.	Growth and spore production good.	Growth rate high, no staling, spore production depressed.
Asbolisia sp...	Growth thin, pycnidium production medium.	Growth and pycnidium production good.	Growth rate depressed on $NaNO_3$ agar, medium on peptone agar, no staling, pycnidium production poor on $NaNO_3$ agar, medium on peptone agar.
Cladosporium herbarum ..	Growth thin, spore production good.	Growth and spore production good.	Growth rate high, no staling, spore production good on $NaNO_3$ agar, poor on peptone agar.
Dematium pullulans ..	Growth thin and white on $NaNO_3$, slow and dark on peptone agar.	Growth good, colony greyish green on peptone, white on $NaNO_3$ agar.	Growth rapid on peptone, slow on $NaNO_3$ agar, colony greyish on peptone, white on $NaNO_3$ agar.
Caldariomyces sp.	Growth thin, spore production good.	Growth and spore production good.	Growth rate and spore production depressed, no staling.
Capnodium sp.	Growth thin, pycnidium production good.	Growth and pycnidium production good.	Growth rate and pycnidium production depressed, staling.
Microxyphium sp. A ..	Growth and pycnidium production good.		Growth rate depressed, pycnidium production prevented.
Microxyphium sp. B	Growth rate slightly depressed.	Growth good.	Growth rate depressed.
Microxyphium sp. C ..	Growth and pycnidium production good.		Growth rate depressed, staling, pycnidium production prevented.

TABLE 2.

Carbohydrate-Nitrogen Ratio High.		Carbohydrate-Nitrogen Ratio Low.	
Carbohydrate medium, Nitrogen low.	Carbohydrate high, Nitrogen medium.	Carbohydrate low, Nitrogen medium.	Carbohydrate medium, Nitrogen high.
Growth thin, spore production medium.	Growth and spore production good.	Growth thin, spore production medium.	Growth and spore production good.
Growth thin, spore production good.	Growth rate slightly retarded, spore production good, no staling.	Growth thin, spore production good.	Growth rate slow, spore production poor on dextrose $NaNO_3$ agar, staling on peptone and maltose $NaNO_3$ agars.
Growth thin, pycnidium production poor.	Growth very rapid, pycnidium production good, no staling.	Growth thin, pycnidium production poor.	Growth slow, pycnidium production depressed, staling on peptone agar.
Growth thin, spore production medium.	Growth slow, no staling, spore production good.	Growth thin, spore production good.	Growth rate and spore production depressed.
Growth thin, colony white.	Growth very rapid, especially on peptone agar, no staling, colony white.	Growth thin, colony white.	Growth slow, staling marked, especially on peptone agar, colony white.
Growth thin, spore production good.	Growth good, spore production slightly depressed, no staling.	Growth thin, spore production good.	Growth medium, spore production depressed, staling on peptone agar.
Growth thin, pycnidium production good.	Growth good, pycnidium production slightly depressed, no staling.	Growth thin, pycnidium production good.	Growth slow, pycnidium production depressed, staling on peptone agar.
Growth thin, pycnidium production good.	Growth good, pycnidium production depressed.	Growth thin, pycnidium production good.	Growth good, pycnidium production depressed, staling.
Growth rate depressed, no staling.		Growth medium.	Growth rate depressed.
Growth and pycnidium production good.	Growth rate depressed, pycnidium production prevented, no staling.	Growth and pycnidium production good.	Growth rate depressed pycnidium production prevented, staling.



Text-figs. 29-36.—Graphs to show growth rates.

29.—*Cladosporium herbarum* on sodium nitrate agar with different concentrations of sugar. Sugar concentration low (1), medium (2), high (3).

30.—*Dematium pullulans* on peptone agar with different concentrations of sugar. Sugar concentration low (1), medium (2), high (3).

31.—*Caldariomyces* sp. on sodium nitrate agar with different concentrations of sugar. Sugar concentration low (1), medium (2), high (3).

32.—*Microxyphium* sp. B on sodium nitrate agar with different concentrations of sugar. Sugar concentration low (1), medium (2), high (3).

33.—*Penicillium expansum* on dextrose (D) and maltose (M) agars of varying sodium nitrate concentration. Nitrogen concentration low (1), medium (2), high (3).

34.—*Penicillium expansum* on dextrose (D) and maltose (M) agars with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

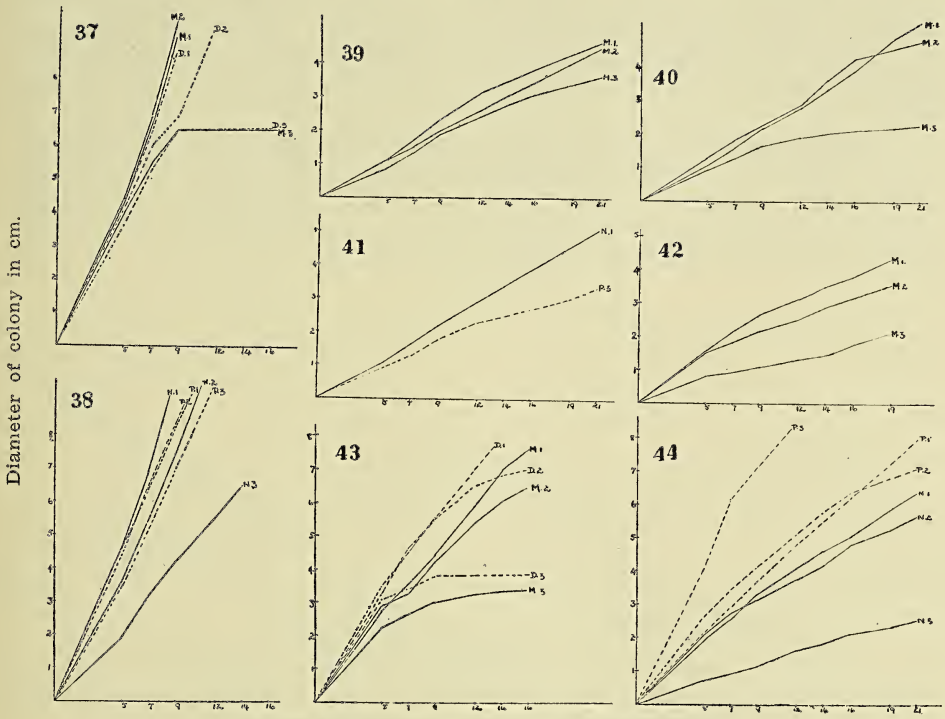
35.—*Penicillium expansum* on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

36.—*Asbolisia* sp. on dextrose (D) and maltose (M) agars of varying sodium nitrate concentration. $NaNO_3$ concentration low (1), medium (2), high (3).

and 34 are compared with Text-figure 35 it can be seen that, although staling is marked at high concentrations of nitrogen when the sugar concentration is medium, no staling occurs in media in which the concentration of sugar as well as that of nitrogen is high. The growth rate is greatly increased in media of high peptone, high sugar concentration, but the spore production is much reduced. Spore production is good on media of high sugar, high $NaNO_3$ concentration.

Asbolisia sp.—The results obtained with this fungus resemble those obtained for *Penicillium*. Growth and pycnidium production are best at moderate concentrations. The actual diameter of the colony may be greater on media of low

concentrations, but the growth is thinner and the pycnidia fewer. On $NaNO_3$ agars growth at high concentrations is slow and no pycnidia are produced. No definite staling, however, is indicated (Text-fig. 36). Staling at high concentrations of peptone is very marked (Text-fig. 37). Pycnidia are produced on dextrose agar but not on maltose agar. As in the case of *Penicillium*, when the concentrations of both peptone and sugar are high no staling takes place, and pycnidium production



Text-figs. 37-44.—Graphs to show growth rates.

37.—*Asbolisia* sp. on dextrose (D) and maltose (M) agars of varying peptone concentration. Peptone concentration low (1), medium (2), high (3).

38.—*Asbolisia* sp. on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

39.—*Cladosporium herbarum* on maltose agar with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

40.—*Cladosporium herbarum* on maltose agar with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

41.—*Cladosporium herbarum* on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentrations low (1), medium (2), high (3). The curves representing growth on media of low, medium and high $NaNO_3$ concentration, and of high and medium peptone concentration, are similar. Accordingly only the curves for low dextrin, low $NaNO_3$ and high peptone concentration are given.

42.—*Dematium pullulans* on maltose agar with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

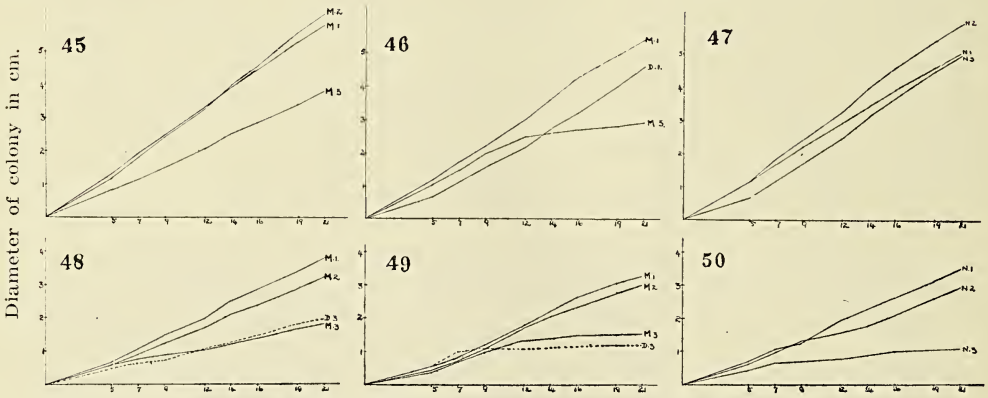
43.—*Dematium pullulans* on dextrose (D) and maltose (M) agars with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

44.—*Dematium pullulans* on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

is satisfactory. In the corresponding experiment with high $NaNO_3$ and sugar concentrations, the growth rate is slow and pycnidia are not produced (Text-fig. 38).

Cladosporium herbarum.—Text-figures 39, 40 and 41 indicate results similar to those obtained for *Penicillium*. Growth and spore production are best on media of medium concentration. Fruiting is inhibited at high concentrations of peptone and is poor at high concentrations of $NaNO_3$ on both dextrose and maltose media. Staling is marked at high concentrations of peptone (Text-fig. 40), but is less marked at high concentrations of $NaNO_3$ (Text-fig. 39). The growth rate is high and there is no evidence of staling when the concentrations of sugar and nitrogen are both high (Text-fig. 41). Spore production is depressed especially on peptone agar.

Dematium pullulans.—Results were similar to those obtained for *Cladosporium*. Staling takes place at high concentrations of peptone (Text-fig. 43), but growth is rapid and no staling is shown when the sugar concentration, as well as the peptone concentration, is high (Text-fig. 44). At high concentrations of $NaNO_3$ growth is slow, but staling is not shown (Text-fig. 42).



Text-figs. 45-50.—Graphs to show growth rates.

45.—*Caldariomyces* sp. on maltose agar with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

46.—*Caldariomyces* sp. on dextrose (D) and maltose (M) agars with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3). The curve representing growth on maltose agar of medium concentration of $NaNO_3$ is similar to that on maltose agar of low $NaNO_3$ concentration. The curve representing growth on dextrose agar of low $NaNO_3$ concentration is similar to that on dextrose agar of medium $NaNO_3$ concentration. The curve representing growth on maltose agar of high $NaNO_3$ concentration is similar to that on dextrose agar of high $NaNO_3$ concentration.

47.—*Caldariomyces* sp. on sodium nitrate agar with different concentrations of sodium nitrate and sugar. Concentration low (1), medium (2), high (3).

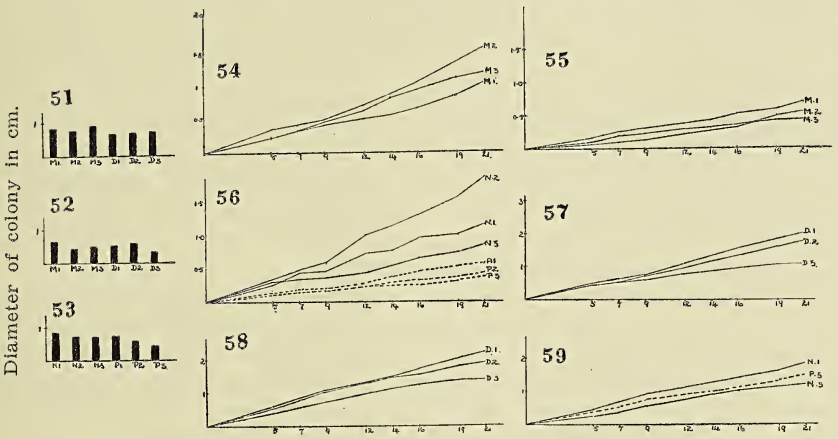
48.—*Capnodium* sp., strain 2, on dextrose (D) and maltose (M) agars with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

49.—*Capnodium* sp., strain 2, on dextrose (D) and maltose (M) agars with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

50.—*Capnodium* sp., strain 2, on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

Caldariomyces sp.—Spore production is poor at high concentrations of peptone and of $NaNO_3$. Staling takes place at high concentrations of peptone (Text-fig. 46) and the fungal colonies are thick and irregular. No staling takes place at high concentrations of $NaNO_3$ (Text-fig. 45). No staling takes place when the concentrations of sugar and of nitrogen are high (Text-fig. 47), but spore production is poor. At low concentrations of nitrogen, and of sugar and nitrogen, growth is thin and spore production very good.

Capnodium sp.—The reactions of both strains are similar. Growth is slow at high concentrations of $NaNO_3$ (Text-fig. 48) and peptone (Text-fig. 49) and pycnidia are not produced. Staling is pronounced, especially on peptone. Staling is also indicated when the concentrations of sugar and nitrogen are both high (Text-fig. 50), and no pycnidia are produced. Growth at low concentrations is thin but satisfactory, and pycnidium production is good.



Text-figs. 51-53.—Graphs to show amount of growth made after 21 days.

51.—By *Microxyphium* sp. A, strain 1, on dextrose (D) and maltose (M) agars with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

52.—By *Microxyphium* sp. A, strain 1, on dextrose (D) and maltose (M) agars with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

53.—By *Microxyphium* sp. A, strain 1, on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

Text-figs. 54-59.—Graphs to show growth rates.

54.—*Microxyphium* sp. B on maltose agar with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

55.—*Microxyphium* sp. B on maltose agar with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

56.—*Microxyphium* sp. B on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

57.—*Microxyphium* sp. C on dextrose agar with different concentrations of sodium nitrate. $NaNO_3$ concentration low (1), medium (2), high (3).

58.—*Microxyphium* sp. C on dextrose agar with different concentrations of peptone. Peptone concentration low (1), medium (2), high (3).

59.—*Microxyphium* sp. C on peptone (P) and sodium nitrate (N) agars with different concentrations of sugar and nitrogen. Concentration low (1), medium (2), high (3).

Microxyphium sp. A.—Staling was not apparent during the course of the experiment on any of the media used. Growth at high concentrations is thick and irregular, but the ultimate diameter of the colonies is much the same at all concentrations (Text-figs. 51, 52, 53). Pycnidia are produced at high concentrations of $NaNO_3$, but not at high concentrations of peptone, nor when the concentrations of sugar and nitrogen are both high. Pycnidia are produced abundantly at low and medium concentrations.

Microxyphium sp. B.—No staling is shown on any of the media (Text-figs. 54, 55, 56). Best growth takes place on media of medium concentration.

Microxyphium sp. C.—Staling is indicated at high concentrations of peptone (Text-fig. 58) and of $NaNO_3$ (Text-fig. 57), but not when the concentration of sugar is high as well as that of the nitrogen (Text-fig. 59). The growth rate at all high concentrations is slow, and no pycnidia are produced. Growth and pycnidium production are good at medium and low concentrations.

From an examination of the above results it is apparent that a low carbohydrate-nitrogen ratio obtained by combining a high concentration of nitrogen with a medium amount of sugar depressed or entirely prevented spore formation in most cases. This effect was more marked with peptone than with $NaNO_3$. The results when a low carbohydrate-nitrogen ratio was obtained by combining a medium concentration of nitrogen with a low concentration of sugar differ from these strongly, and resemble much more closely the results obtained at low concentrations of sugar and nitrogen. It would appear that the reduction in size of the colony and the amount of spore production was due to starvation rather than to the unbalanced carbohydrate-nitrogen ratio.

At high concentrations of peptone and medium concentrations of sugar, staling phenomena are very strongly shown except by the fast growing *Botrytis* and the very slow growing *Microxyphium* spp. A and B. Staling is less marked at high concentrations of $NaNO_3$. Although staling is marked at high nitrogen concentrations, it does not often occur when the concentrations of sugar and nitrogen are both high, i.e., at a balanced ratio of carbohydrate and nitrogen.

A high ratio, due to high concentration of sugar, somewhat depresses the growth rate in all but *Asbolisia*, *Dematium*, *Botrytis* and *Caldariomyces*, and also slightly depresses the spore production. At a high ratio due to low concentration of nitrogen the growth is sparse and the fruiting less in most cases. This again may be due to starvation rather than to an unbalanced ratio of carbohydrate and nitrogen.

Staling of the medium and reduction in the amount of spore production appear to be related to some extent. Staling appears to be a function of the nitrogen compound. This has also been found to be the case by Brown and Horne (1924) and Brown (1925) in the case of species and strains of *Fusarium*.

Both the concentration and the type of compound are of importance, as it has been found that $NaNO_3$ is not so conducive to staling at high concentrations as peptone, and unfavourable nitrogen compounds such as ammonium salts cause staling at low concentrations. Brown and Horne (1924) conclude that staling is also dependent on the concentration of the medium, and that the effects of concentration can be removed by dilution of the media. In the present investigation the effect of staling at high concentrations of the culture medium is strongly marked only in the case of *Capnodium* sp. Its effect was not observed in other cases, possibly because the concentration of the medium was not sufficiently great. It was apparent, however, that a concentration of nitrogen

sufficient to cause serious staling is not effective if the concentration of sugar is increased to balance it.

In agreement with the observations of Horne and Mitter (1927) it was found that high and low concentrations retard the growth rate. It is also apparent that each species has a different optimum concentration and a different range of concentrations suitable for growth. In the Capnodiaceae best growth takes place on media of low and medium concentration, whereas *Dematium* and *Asbolisia* respond to high concentrations of peptone, and *Cladosporium* and *Penicillium* grow more or less satisfactorily on media of high and low concentrations.

In the higher plants it has been shown (Knight, 1924/5; Kraus, 1925) that the relation between the amount of vegetative growth and the amount of reproductive growth made by a plant is determined by the relative amounts of carbohydrate and nitrogenous materials available within it. Efficient growth and reproduction are dependent on a balanced carbohydrate-nitrogen ratio. A low ratio caused by an increase in the amount of nitrogen results in vegetative growth; and a high ratio caused by excess of carbohydrates reduces vegetative growth without inducing fruitfulness.

It appears from the present investigation that much the same relationship holds for the fungi. Spore production is slowed down or prevented by a low ratio due to excess of nitrogenous material, and by an unduly high ratio. Maximum fruitfulness and most efficient vegetative growth take place when the amounts of carbohydrate and nitrogen are balanced and of moderate concentration.

Summary.

1. Experiments have been made to ascertain culturally whether there are any appreciable differences between sooty mould fungi and other moulds in their ability to utilize different classes of food materials.

2. For this purpose representatives of all types of sooty mould fungi, with *Penicillium expansum* and *Botrytis cinerea* as controls, have been grown on agar media containing a variety of different food materials.

3. *Penicillium expansum* and *Cladosporium herbarum* were able to utilize a wide range of carbohydrates. The other fungi utilized the pentoses and mannose and lactose rather less well. Only *Dematium pullulans* and *Botrytis cinerea* were unable to make good growth on the trisaccharide raffinose.

4. All the fungi grew well on the polysaccharides inulin, dextrin and starch.

5. *Penicillium* was able to use the widest range of nitrogenous compounds. *Cladosporium* was able to grow satisfactorily on peptone, KNO_3 and $NaNO_3$ only. Asparagin was generally unsatisfactory and caused pronounced staling. Members of the Capnodiaceae grew moderately well on all the media tested.

6. By varying the amount of nitrogenous and carbohydrate food material in the agar media the reactions of the fungi to changes of the carbohydrate-nitrogen ratio have been studied. It appears that the fungi resemble the higher plants to a certain extent in that fruiting is poor at low ratios when nitrogen is present in high concentration.

7. Staling was pronounced in agars of moderate sugar and high peptone concentration, but much less so in agars with moderate concentration of sugar and high $NaNO_3$. In most cases staling was not shown when the concentration of both sugar and nitrogen was high.

8. Staling and decrease in spore production appear to be related to some extent.

9. Each fungus has a different optimum concentration of carbohydrate and nitrogen, some being able to utilize higher concentrations than others.

In conclusion, the writer wishes to thank Professor T. G. B. Osborn, in whose laboratory this work was carried out, for his interest and helpful criticism.

Literature Cited.

ARNAUD, G., 1911.—Contribution à létude des Fumagines. 2. *Ann. Ecole nationale Agri. Montpellier,* Sér. 2, Tome x (iii/iv), pp. 211-330.

BROWN, W., 1923.—Experiments on the Growth of Fungi in Culture Media. *Ann. Bot.,* xxxvii (cxlv), pp. 105-129.

————, 1925.—Studies in the Genus *Fusarium.* ii. An Analysis of Factors which determine the Growth Forms of Certain Strains. *Ann. Bot.,* xxxix (cliv), pp. 373-408.

————, and HORNE, A.S., 1924.—Studies in the Genus *Fusarium.* i. General Account. *Ann. Bot.,* xxxviii (cl), pp. 379-383.

————, ————, 1926.—Studies in the Genus *Fusarium.* iii. An Analysis of Factors which determine Certain Microscopic Features of Fusarium Strains. *Ann. Bot.,* xl (clvii), pp. 203-221.

FARRIES, E. H. M., and BELL, A. F., 1930.—On the Metabolism of *Nematospora gossypii* and Related Fungi, with Special Reference to the Source of Nitrogen. *Ann. Bot.,* xliv (clxxiv), pp. 423-456.

FRASER, L., 1933.—The Sooty Moulds of N.S.W. i. Historical and Introductory Account. PROC. LINN. SOC. N.S.W., lviii, 375-395.

HORNE, A. S., and MITTER, J. H., 1927.—Studies in the Genus *Fusarium.* v. Factors determining Septation and Other Features in the Section Discolor. *Ann. Bot.,* xli (clxiii), pp. 521-547.

KNIGHT, D., 1924/5.—The Carbohydrate-Nitrogen Ratio. *Science Progress,* Vol. 19, p. 34.

KRAUS, E. J., 1925.—Soil Nutrients in Relation to Vegetation and Reproduction. *Amer. Journ. Bot.,* Vol. 12, p. 510.

NORMAN, A. G., 1930.—The Biological Decomposition of Plant Materials. iii. Physiological Studies on Some Cellulose Decomposing Fungi. *Ann. Appl. Biol.,* xvii (3), pp. 575-612.